be due to multiple genetic alterations resulting in complex changes in expression of many genes. The parental malignant melanoma cell line UACC903 displays anchorage-independent growth and the chromosome 6-mediated suppressed subline Clone1 is anchorage-dependent<sup>1</sup>. The revertant cell line SRS3, derived from Clone1 by retroviral transduction, resembles the phenotype of UACC903 (ref. 2). Here we describe identification of the chromosome 6-encoded tumour-suppressor gene encoding connexin43 (Cx43) by cDNA microarray and suppression of the anchorage-independent growth of UACC903 by overexpression of this gene. We first measured expression of 4,536 genes between UACC903, Clone1 and SRS3 using cDNA microarrays, resulting in 7.08% of genes (321/4,536) showing changes in their expression levels. Notably, 12 genes displayed higher levels of expression in Clone1 than in both UACC903 and SRS3, providing candidates for further identification of tumour-suppressor genes. Genes encoding Cx43 (suppressor activity), monocyte chemotactic protein1 (MCAF/MCP1; suppressor activity) and cysteine proteinase CPP32a (apoptotic activity) were all upregulated in Clone1 in contrast to both UACC903 and SRS3. Transfection of Cx43, encoded on chromosome 6q21-q23, a region frequently altered in malignant melanoma, resulted in its overexpression and suppression of anchorage-independent growth of UACC903. Thus, our results demonstrate that the combination of the ability to alter cellular phenotype by successive genetic alterations and the ability to examine the gene expression patterns facilitates identification of a tumour-suppressor gene.

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## Syvänen, Ann-Christine

## Primer extension on microarrays for typing single-nucleotide polymorphisms and detecting disease-causing mutations

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In the 'minisequencing' primer-extension method, the DNA synthesis reaction catalysed by the DNA polymerases is used to distinguish between sequence variants<sup>1</sup>. We have converted the mini-sequencing reaction principle into a microarray format, and shown experimentally that it discriminates more than tenfold better between homozygous and heterozygous genotypes than hybridization with allelespecific oligonucleotide probes in the same array format<sup>2</sup>. The minisequencing microarrays are manufactured by coupling 1 or 2 primers per mutation to be detected as 100-M spots at a density of 400 M on a glass surface using an 'inhouse'-constructed printing robot. The arrayed primers are allowed to anneal to templates amplified by multiplex PCR, and the polymorphic nucleotides are detected by extending the primers with labelled nucleotide analogues using a DNA polymerase. For reading the fluorescence signals on the microarrays, we use an in-house experimental multicolour fluorescence scanner or the commercial 'ScanArray' instrument. The genotypes are assigned by simple calculation of the ratios between the signals for normal and mutant nucleotides. We have applied our array-based minisequencing system to analyse SNPs in a set of candidate genes for their association with myocardial infarction in a case-control study in the Finnish population3. We found that allelic variants of PAI1 and GPIIIa genes contributed an additive risk of developing myocardial infarction. As an alternative to minisequencing, we have developed a method based on allele-specific extension of immobilized detection primers. We are using this method to screen regional population samples from Finland for 30 mutations of the 'Finnish disease heritage' and other recessive diseases to determine their population frequencies and to identify regional clusters of disease carriers. All the mutations, constituting both point mutations and small and large deletions, are distinguished unequivocally in both heterozygous and homozygous form by our fluorescence-based primer extension assay on microarrays. The fact that we are able to use our inhouse genotyping system based on primer arrays of intermediate density in practice to generate tens of thousands genotypes is evidence for the robustness of the primer extension approach. Moreover, the specific primer extension reactions on the microarrays allows quantitative multiplex mutation detection. The through-put of the system is only limited by the capacity of performing multiplex PCR amplifications. Based on our results, we are convinced that primer extension is a more promising reaction principle for future efficient genotyping on high-density DNA chips than hybridization-based approaches.

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## Tao, Lian

## Isolation of chromosome 6-encoded differentially expressed genes associated with breast cancer cell lines MDA-MB-231 and MDA/H6 by cDNA microarray

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The loss of heterozygosity and chromosomal alterations were frequently observed on human chromosome 6 in cancers. In this study, we analyse the relative RNA levels between the parental breast cancer cell line MDA-MB-231 and the chromosome 6-mediated suppressed cell subline MDA/H6 using high-density gene filters containing more than 5,000 genes. Two cell lines were cultured under the same growth conditions for RNA preparation. Total RNA was reverse transcribed into first-strand cDNA in the presence of a<sup>33</sup>P-dCTP. Approximately 10E8cpm of the labelled cDNAs were used for hybridization. Signal intensities from the hybridization were used to compare the relative levels of gene expression. Our analysis revealed approximately 100 genes were differentially expressed between the two cell lines. We identified 10 differentially expressed genes encoded on human chromosome 6, including genes encoding insulin-like growth factor 2 receptor, 5T4 oncofetal antigen and protein-tyrosine kinase 7. These results should facilitate our understanding of the molecular basis of the chromosome 6mediated suppression of breast cancer.